EXAGGERATED ELEVATION OF THE FERTILIZATION MEMBRANE OF CHAETOPTERUS EGGS, RESULTING FROM COLD-TREATMENT[1]

CATHERINE HENLEY

Marine Biological Laboratory, Woods Hole, Mass., and Department of Zoology, University of North Carolina, Chapel Hill, N. C.

There are many phenomena associated with elevation of the vitelline membrane after activation of invertebrate eggs; these have long been of interest to embryologists. Recently, Costello (1958a) has summarized much of the earlier work, and has contributed new observations concerning the behavior of the vitelline membrane and the jelly material which is secreted by the egg of *Nereis limbata* after fertilization. These studies involved the centrifuging and subsequent treatment of *Nereis* eggs with alkaline sodium chloride (pH 10.5), which had earlier been shown by Costello (1945) to be effective in producing exaggerated membrane elevation and denuding of the eggs. The method is based upon one first described by Hatt (1931) and subsequently modified by Novikoff (1939) for the eggs of *Sabellaria vulgaris*.

Costello (1958a) interpreted his results to indicate that centrifuging of the *Nereis* egg resulted in a displacement of the cortical jelly-precursor material, so that upon subsequent activation such centrifuged eggs had an asymmetrical perivitelline space which was widest at the centrifugal pole. The jelly-layer, which forms external to the membrane, was also asymmetrical and thickest at the same area. When centrifuged unfertilized *Nereis* eggs were treated with alkaline NaCl, there was a retention of the jelly beneath the vitelline membrane and formation of an asymmetrical perivitelline space.

Activation of the egg of *Chaetopterus pergamentaceus* is not accompanied by cortical changes of so obvious a nature as those reported for *Nereis* by Lillie (1911), Novikoff (1939) and Costello (1949, 1958a). There are, however, inconspicuous rhythmic contractions of the vitelline membrane of the *Chaetopterus* egg, beginning about 20 minutes after fertilization, which were described briefly by Lillie (1906) and in more detail by Pasteels (1950). It was suggested by Costello (1958a) that (page 180), "These might be occasioned by rhythmic release of small quantities of colloidal material in progressive waves sweeping over the egg surface from an origin at one pole or the other." Evidence which offers support for this hypothesis has been obtained during the course of experiments involving the treatment of *Chaetopterus* eggs with low temperature.

Brief preliminary accounts of some of this work have been reported (Costello and Henley, 1949; Henley and Costello, 1949; Henley, 1958).

[1] Aided by a grant from the National Institutes of Health, U. S. Public Health Service, RG-5328, to Dr. D. P. Costello. I am indebted to Dr. Costello for a critical reading of the manuscript, and for his assistance with the photographs.

Methods

Eggs and sperm of the polychaete annelid *Chaetopterus pergamentaceus* were obtained and handled by the methods outlined by Costello *et al.* (1957). The general plan of the experiments involved the following procedure: Two to five minutes after insemination, the experimental eggs were, for those series which involved temperature shock, transferred to six-inch fingerbowls containing pre-chilled (2–3° C.), freshly filtered, aerated sea water; the fingerbowls rested in a nest of cracked ice contained in a large fingerbowl, which in turn was kept in the refrigerator. In the series of experiments which did not involve temperature shock, the eggs (contained in a six-inch fingerbowl of freshly filtered, aerated sea water at room temperature) were gradually chilled by placing the fingerbowl in a larger dish of cracked ice which in turn was placed in the refrigerator. Usually a period of about 60 minutes was required for the temperature of 2–3° C. to be reached in those experiments which did not involve temperature shock.

Appropriate controls were kept for all series; these dishes were kept surrounded by running sea water.

At the end of the treatment period, the culture dishes in all series were removed from the refrigerator and ice-bath to the sea water table, and allowed to return gradually to room temperature, which varied from 20 to 24.5° C. during the various periods of the experiments. Egg-samples were removed and studied at frequent intervals during and following the warming-up period. No temperature shock was involved during the post-treatment period for any of the experiments.

Counts were made to determine the approximate time of 50% cleavage in experimental and control egg-populations, so that a quantitative measure could be obtained of the cleavage delay brought about as a consequence of treatment. All observations and photographs of living eggs and embryos were made without the use of coverslips; drops of culture fluid were examined in uncovered Columbia watchglasses.

For some series, fixed and stained whole-mount preparations were made of control and experimental eggs. These were prepared according to the method described by Henley and Costello (1957), being fixed in Kahle's fluid and stained in Harris' acid haematoxylin, or by the Feulgen technique. Most of the results reported here, however, are based upon observations of living embryos and larvae.

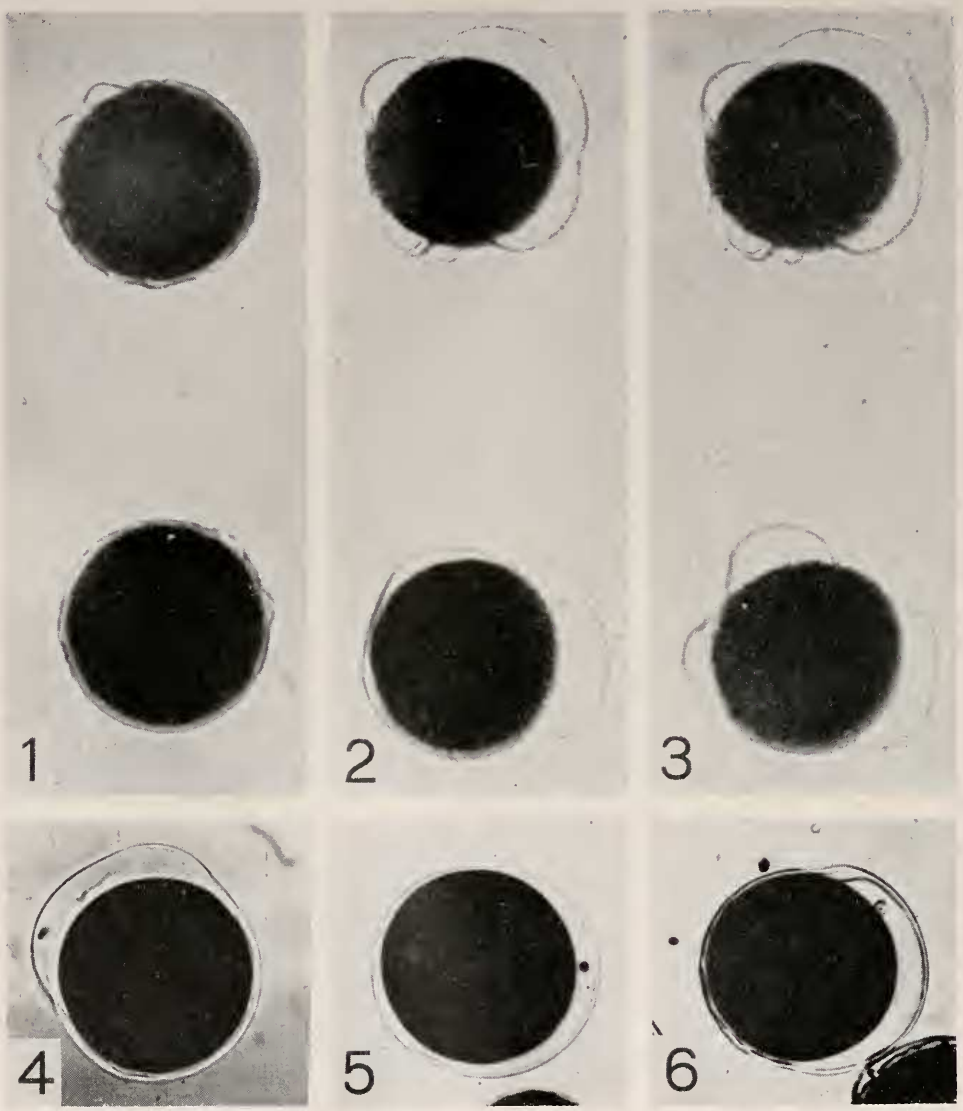
Photographs of living and fixed eggs were made using a Leica camera with Micro-Ibso attachment.

A total of over 30 experiments was performed, during the course of several summers at Woods Hole.

Results

Membrane elevation

The most striking single result of cold-treatment of *Chaetopterus* eggs is the markedly asymmetrical exaggeration of vitelline membrane elevation, which occurs during the period of gradual return of the eggs to room temperature (Figs. 1–10). This process begins rather slowly and, for the first 10 minutes or so (after moderately short periods of treatment), appears to involve no atypical changes in the egg or membrane. A relatively localized shallow crinkling of the membrane then begins at one sector (Fig. 1, lower egg); this is probably, at most, only a slight



All photomicrographs are of living, fertilized *Chactopterus* eggs; the pictures were made with a Leica camera and Micro-Ibso attachment. Magnification of all figures after reproduction: approximately 250 ×.

FIGURE 1. Eggs cold-treated for 150 minutes with temperature shock and photographed 11 minutes after the end of treatment. In the lower egg, the first area of membrane wrinkling is visible at approximately one o'clock on the egg periphery; the elevation of the entire membrane is still at about the normal height. In the upper egg, the second area of membrane "blistering" has begun to appear, the original area of crenation still being visible at the lower pole of the egg.

FIGURE 2. Another pair of eggs, cold-treated for 150 minutes with temperature shock and photographed 21 minutes after the end of treatment. Note the enlarged "blistered" areas in the membranes of both eggs.

exaggeration of the normal membrane wrinklings which occur at the vegetal pole, beginning about 20 minutes after insemination (Lillie, 1906; Pasteels, 1950). Within a period of approximately five minutes, a second, much more pronounced, localized area of membrane elevation appears in the treated eggs (Fig. 1, upper egg). This second region is the one from which subsequent exaggerated membrane elevation proceeds. It is, from its first appearance, quite distinctive and is characterized by a more blister-like configuration of the membrane (Fig. 2). It appears to bear no constant spatial relationship to the original wrinkled vegetal pole area from which, as aforesaid, it is entirely separate.

During the course of the next few minutes, additional deep folds may appear in the membrane (Fig. 3); eventually these become contiguous and by 35 minutes after the end of treatment (approximately equivalent to 40 minutes after insemination) the membrane is smoothly and very exaggeratedly elevated from the egg surface (Fig. 10, upper egg). The course of membrane elevation during the period from 28 minutes to 33 minutes after the end of treatment is shown in Figures 7–10. These pictures show clearly that once the process of exaggerated membrane elevation begins, it proceeds rapidly (see also Figures 2 and 3, photographs taken one minute apart).

The ultimate exaggerated elevation attained may be represented by Figure 10 (top), shown 33 minutes after the end of treatment. The elevation of the membrane is quite markedly asymmetrical, the point where it is nearest the egg surface representing the sector which was first wrinkled (not that second center from which the process of elevation proceeds). Even here, however, at the point of closest approach to the egg surface, the elevation of the membrane is much wider than normal. The asymmetrical nature of this elevation is a characteristic and consistent result of cold-treatment.

Comparison of Figures 1 (lower egg) and 10 (upper egg) will reveal the magnitude of the exaggerated membrane elevation; although Figure 1 represents a treated egg, the elevation of the membrane has not yet proceeded beyond the normal stage.

It is important to note that except for the first area of shallow membrane wrinkling, the subsequent stages are probably quite separate and distinct (in degree, at least) from the normal membrane shape changes described by Pasteels (1950). His description of the initial crenated area in an egg 20 minutes after insemination

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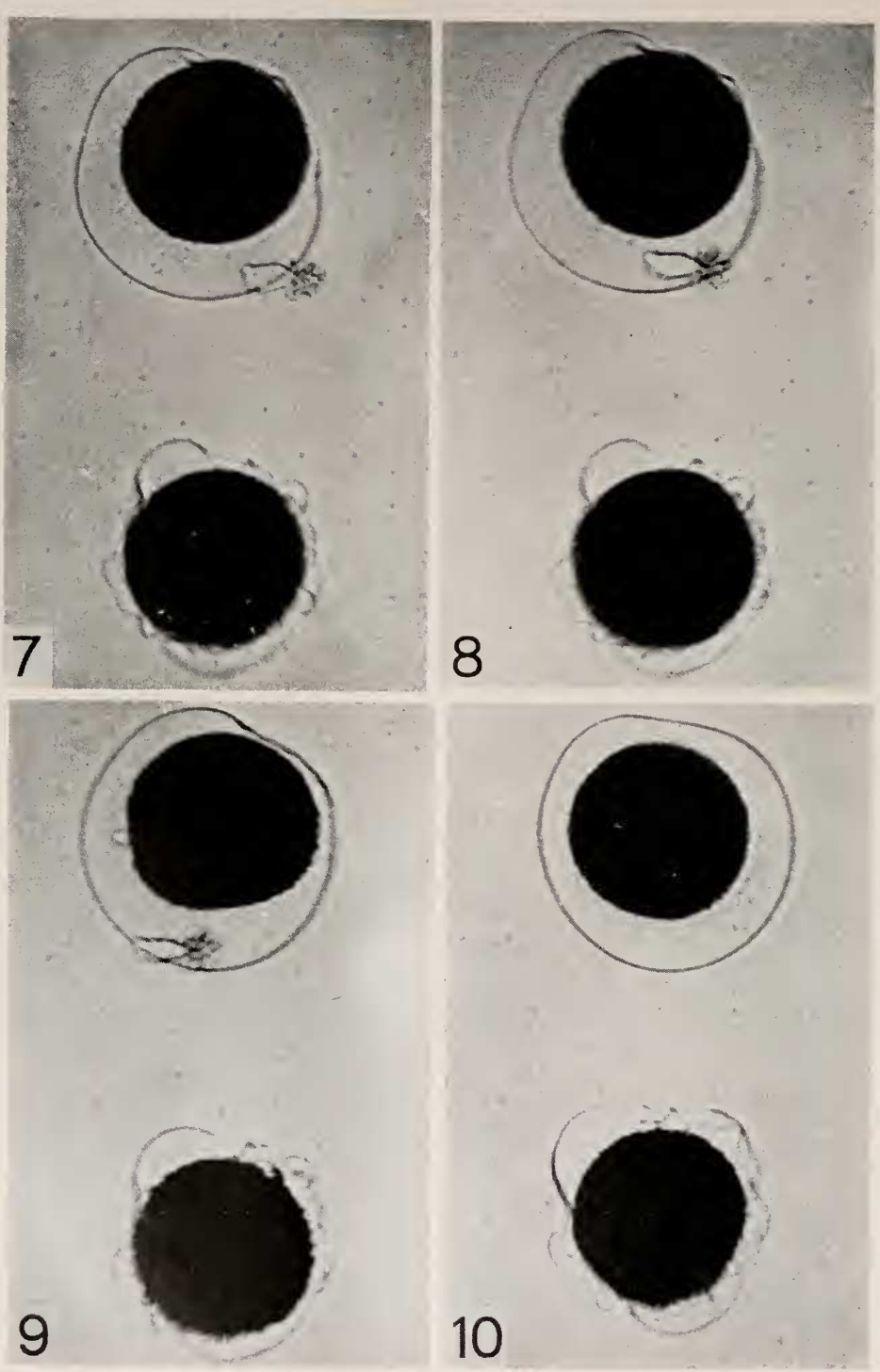
FIGURE 3. The same pair of eggs shown in Figure 2, photographed 22 minutes after the end of treatment (one minute after the photograph of Fig. 2). Note that two additional localized areas of exaggerated membrane elevation have now appeared in the lower egg.

FIGURE 4. Egg cold-treated for 6 hours without temperature shock and photographed immediately after the end of treatment. In this case, the process of exaggerated membrane elevation has begun considerably sooner after treatment than usual. The localized character of the elevation and the double nature of the membrane are apparent.

FIGURE 5. Another egg from the same treatment group as that shown in Figure 4, but photographed 50 minutes after the end of treatment. The double nature of the exaggeratedly elevated membrane is visible. The small globule between the egg surface and the membrane at the right is apparently a polar body.

FIGURE 6. A third egg from the same experimental group as those shown in Figures 4 and 5, photographed 50 minutes after the end of a 6-hour cold-treatment. The membrane has remained closely apposed to the egg surface around approximately one-half of the egg periphery but is exaggeratedly elevated around the other half. The double nature of the membrane is clear.



FIGURES 7–10.

(at an undesignated temperature) as having a "finement plissé" appearance is very accurate, and his Figure A shows that the subsequent normal wrinklings and shape changes are likewise quite shallow. We have repeatedly confirmed his findings in the study of normal, control eggs, and they are also illustrated in the photographs accompanying the paper by Harvey (1939).

There is no way of knowing with certainty whether the changes observed in our treated eggs are in any way comparable to the normal wrinklings, since the exaggerated membrane elevation is so drastic as to obscure any crenations of the type seen in untreated eggs. They are not associated with the presence of a coverslip, since all our observations of living eggs were made without the use of a coverslip. Careful study of living eggs, of fixed whole-mount preparations, and of photographs of living eggs has convinced us that the exaggeratedly elevated membrane is double in nature (Figs. 4–6).

Cleavage delay

At room temperatures of 20–25° C., the first cleavage of *Chaetopterus* eggs normally occurs from 40 to 50 minutes after insemination (Costello *et al.*, 1957). In the cold-treated eggs studied here, first cleavage for 50% of the experimental eggs was delayed from 134 to 704 minutes, the magnitude of the delay being in direct relation to the duration of treatment (Table I). As noted below in the section on the role of temperature shock, cleavage delay was much greater in those eggs subjected to temperature shock than in those which were cooled gradually (Table II), in series treated for the same length of time. From cytological study, it appears that the cold-treated eggs did not develop very far after the onset of chilling, until the treatment was ended and a gradual warming process (to room temperature) began. During the five-minute period which intervened between the time of insemination and the time when treatment was begun, the sperm penetrated the egg and, in some cases at least, the male and female pronuclei approached one another. Further development did not occur until the conclusion of the treatment. From the data reported in Table II, it appears that a period of from three to ten minutes was required for resumption of development in all cases where eggs were treated with temperature shock. For those eggs which were not abruptly chilled, there was apparently a continuation of some of the early stages of development, during the start of the cooling process, so that the magnitude of the cleavage delay was sometimes slightly less than the duration of treatment.

It is of considerable interest that even in those eggs treated for 720 minutes (12 hours), development could be resumed after cessation of the cold-treatment. As noted in Table I, the trochophore larvae developing from eggs treated for this period were very abnormal, however.

FIGURES 7–10. Successive stages of a single pair of eggs, cold-treated for 150 minutes with temperature shock. FIGURE 7: 28 minutes after the end of treatment. Note the pronounced asymmetry of exaggerated elevation in this and the succeeding photographs. FIGURE 8: 29 minutes after the end of treatment. The small particle of debris has shifted position in the interval between this photograph and that of Figure 7. FIGURE 9: 30 minutes after the end of treatment. FIGURE 10: 33 minutes after the end of treatment. The upper egg (unconfined by a coverslip) has rolled over since the photograph of Figure 9 was taken. The small piece of debris has been obscured. See text for further details.

TABLE I

The effects of cold treatment (5–12 hours) on fertilized Chaetopterus eggs

Duration of treatment	Effects on membrane elevation	Cleavage delay for 50% of exper. eggs as compared with controls	Other effects
300 minutes	Variable; some exaggeration by 15 minutes after end of treatment	301–312 minutes	Ciliary defects in trochophores. First cleavage products equal in size?
360 minutes	Ca. 90% with exaggerated membrane elevation immediately after end of treatment	320–346 minutes	Abnormal trochophores; ciliary defects; surface blebs
390 minutes	Exaggerated elevation by 45 minutes after end of treatment; eventual denuding of many eggs	429 minutes	First cleavage products equal in size?
420 minutes	94% of eggs with exaggerated elevation within 5 minutes after end of treatment	*	Abnormal, amorphous trochophores
540 minutes	90% of eggs with exaggerated elevation within 25 minutes after end of treatment	590 minutes	
720 minutes	44% of eggs with exaggerated elevation within 23 minutes after end of treatment	704 minutes	Abnormal trochophores; some very small. Ciliary defects

All treatments were begun within 2–5 minutes after insemination. The sea water medium was gradually chilled to a temperature of 2–3° C. (no temperature shock).

* The cleavages in this group of eggs were very abnormal and chaotic, and could not be timed.

Other effects of low temperature on development

The embryos and trochophores developing from the cold-treated eggs exhibited a number of characteristic abnormalities. In many of the cases where prolonged periods of treatment were used, there appeared to be a suppression of polar body formation in at least some of the eggs. This is in accord with the findings of other investigators, for other forms (notably amphibians; see the review by Fankhauser,

TABLE II

Treatments of comparable durations, with and without temperature shock

Duration of treatment	Temp. shock?	Effects on membrane elevation	Cleavage delay
180 minutes	No	None, or at most very slight	162 minutes
150 minutes	Yes	Asymmetrical exaggerated membrane elevation by 15–20 minutes after the end of treatment	152–160 minutes
120 minutes	Yes	Asymmetrical exaggerated membrane elevation by 25 minutes after the end of treatment	134 minutes
240 minutes	No	Slight exaggeration of membrane elevation in some eggs	220 minutes
185 minutes	Yes	Many eggs with exaggerated membrane elevation by 45 minutes after the end of treatment	189 minutes

1945). Less drastic exposures to low temperature apparently had no effect on polar body formation, so far as could be determined.

In a few experiments, the two blastomeres resulting from the first cleavage of treated eggs were equal or nearly equal in size. (Normally the AB and CD blastomeres resulting from the first cleavage of *Chaetopterus* and other spirally cleaving eggs are noticeably unequal.) Tyler (1930) also observed equal cleavage after cold-treatment of *Chaetopterus* eggs, but his experiments are not entirely comparable to those described in the present experiments, since he used somewhat higher temperatures, applied later in development and for considerably shorter periods of time. Tyler (1930) discusses the production of double embryos by such treatments; no evidence of double embryos, nor of the alteration of cleavage planes as described by him, was observed in the present study.

The normal egg-shape changes ("pear" and polar lobe stages) were often not clearly identifiable in the treated eggs.

The trochophore larvae which developed from cold-treated eggs almost invariably moved very sluggishly, if at all, and there were apparently severe ciliary defects. Surface blebs were often present on the trochophores. In general, the larvae bore a striking resemblance to those obtained after KCl-treatment by Lillie (1902), and after cold-treatment (10–14° C. for 14 hours) by Lillie (1906). However, we do not think that the trochophores in our cultures resulted from differentiation without cleavage, as Lillie (1906) suggested, since cytological study (see below) revealed that at least a degree of both karyokinesis and cytokinesis had occurred in all observed cases.

There was a wide variety of size among trochophores developing from eggs cold-treated for prolonged periods (12 hours). Some larvae were very small, suggesting that they might have developed from egg fragments. Such fragments, or detached cells from later cleavage stages, were observed in culture dishes of this series.

In almost all experiments there was a high mortality rate among the later embryos and trochophore larvae of the experimental groups.

Denuding of eggs

After many of the longer durations of treatment (6 hours or more), the process of exaggerated membrane elevation continued until there was a bursting of the membrane and denuding of the eggs. This denuding was very similar in course and end-results to that observed after alkaline NaCl treatment of *Nereis* and *Sabellaria* eggs. It is, however, in contrast to the process of denuding which follows alkaline NaCl treatment of *Hydroides* eggs (Costello, 1958b); there appears to be an actual dissolution of the *Hydroides* egg membrane in the alkali. (It is suggestive in this connection that Lillie, 1902, described a similar destruction of the *Chaetopterus* egg vitelline membrane in KCl and $CaCl_2$ solutions.) Subsequent development of the denuded *Chaetopterus* eggs was very abnormal under the conditions of these experiments, but no special efforts were made to cultivate such embryos further. Costello (1945) and others have shown that denuded eggs are extremely sensitive to contact with bare glass surfaces, and coating of the culture dishes with a thin layer of agar in sea water is necessary for the successful maintenance of such embryos.

After less drastic treatments, the asymmetrical exaggerated membranes retained their configuration through at least the first cleavage and denuding did not occur.

The role of temperature shock

Table II describes the results of experiments involving comparable durations of treatment time, with and without temperature shock. Even relatively short periods of cold-treatment (120 minutes) involving rapid transfer of the eggs from an ambient medium at room temperature to one pre-chilled to 2–3° C. were effective in producing a marked delay of the first cleavage, as well as the characteristic exaggerated membrane elevation. In contrast, cold-treatments of as long as 240 minutes without this temperature shock resulted in, at most, very slight effects on cleavage time and on membrane elevation. Thus, in one experiment, a treatment of 180 minutes without temperature shock resulted in a delay of 162 minutes for the first cleavage and produced only slight effects on membrane elevation. A similar treatment of 185 minutes with temperature shock resulted in a 189-minute delay of the first cleavage time for 50% of the experimental eggs (as compared with 50% of the controls); by 45 minutes after the end of treatment, there was a marked exaggeration of membrane elevation in many of the treated eggs.

Temperature shock involves the beginning of action of cold considerably sooner after insemination (five minutes) than the absence of temperature shock, where at least 60 minutes may be required to attain the treatment temperatures of 2–3° C. This suggests that the effective action of cold in producing exaggerated membrane elevation occurs within the first hour of treatment; in a gradually cooling medium, there may be opportunity for at least a semblance of the normal release of cortical material to occur, whereas this release is inhibited almost immediately if the eggs are plunged into pre-chilled sea water five minutes after insemination.

Even treatment without temperature shock, however, results in abnormal trochophores and ciliary defects, despite the fact that there may be little or no membrane exaggeration. The implication here is that the effects on membrane elevation may be very different from those affecting subsequent morphogenesis. The low temperature may thus be said to have both a direct and a delayed type of action.

Study of fixed eggs

In one typical group of eggs, cold-treated for 150 minutes with temperature shock, and fixed 15 minutes after the conclusion of the treatment (170 minutes after insemination), the normal quota of 9 chromosomes at the metaphase of the first maturation division was present and countable in most instances. In some eggs, the sperm nucleus was still visible in the interior of the egg, as a separate entity; in others, approach and fusion of the pronuclei had advanced further, and the male and female components were no longer separable on the basis of appearance. Control eggs fixed at the same time were proceeding from the eight- to the sixteen-cell stage in a normal fashion. Experimental eggs of the same series fixed 205 minutes after insemination (50 minutes after the end of treatment) were at the metaphase of the first cleavage, while control eggs were at the metaphase of the fifth cleavage. Several multipolar spindles were observed among the experimental eggs of this group.

The cortical regions of the experimental eggs fixed 50 minutes after the end of treatment presented a striking picture. There was a marked asymmetry of cortical material, apparent as a more lightly staining peripheral band with its greatest width within one relatively localized sector of the egg circumference. These eggs were fixed, whole, on coverslips and there is inherent in the method a certain minor degree of distortion of the egg shape. However, we observed no comparable asymmetry of the cortical material in control eggs of this or any other series, and therefore suggest tentatively that this may represent the area where cortical colloid material has been released in the observed asymmetrical fashion. The eggs of this group were marked by a high incidence of asymmetrical membrane elevation, which was first apparent about 30 minutes after the end of treatment, or 20 minutes before the eggs were fixed. The asymmetry of the cortical area is not visible in the experimental eggs which were fixed 15 minutes after the end of treatment; these ova, in the living condition, had not yet undergone exaggerated membrane elevation.

In addition to the multipolar spindles mentioned above, a number of other types of cytological abnormality were noted, especially in those eggs which were fixed several hours after the end of treatment. Among the abnormalities were chromosome bridges, lagging or lost chromosomes or chromosome fragments, and unequal division of chromatin material to the two poles of the division figure. All these anomalies are typical of the kinds produced in other material as a consequence of low (and high) temperature, among other agents.

There was some evidence of the production of polyploidy in larvae from cold-treated eggs; although a few of the body cells of trochophores obviously had more than the diploid number (18) of chromosomes present, such duplications of individual chromosomes or sets of chromosomes was apparently not the rule in all cells of a given larva. The chromosomes of *Chaetopterus* are small and tend to be crowded on the spindle, so that counting them is difficult, even in early cleavage stages; to do so is almost impossible in the minute cells of later cleavage stages and trochophores. The observed anomalies of chromatin distribution may have arisen as a result of the polar body suppression mentioned above.

Cytological preparations of advanced cleavage stages of the treated eggs indicate that there was often some suppression of cytokinesis (although karyokinesis had proceeded in a variable fashion). In all cases studied, however, at least some degree of cytoplasmic division had occurred, and there was no evidence of differentiation without cleavage. As would be expected, there was a wide variation in cell size, in the treated embryos and larvae.

Discussion

The possible mechanism of action of low temperature

The characteristic asymmetry and exaggeration of membrane elevation in the cold-treated *Chaetopterus* eggs suggest that cold-treatment may interfere with the gradual and rhythmic release of some substance from the egg surface after insemination, so that a sudden localized release of such material occurs at the cessation of treatment. There may also be a change in the permeability of the vitelline membrane, so that the colloidal material is retained within the perivitelline space, bringing about the observed exaggerated membrane elevation. The asymmetry of this

exaggerated elevation could be a consequence of the accumulation of colloidal substance within one relatively localized sector of the egg, or of changes in the egg cortex resulting in release of such a substance in a considerably smaller segment of the egg periphery than is normal. The *Chaetopterus* egg secretes no external jelly after activation, and the substance whose abrupt release brings about membrane elevation in the treated eggs is therefore postulated to be of some other nature. Thus, the situation for the *Chaetopterus* egg differs, in detail at least, from that described for the *Nereis* egg by Costello (1958a).

Some additional evidence for this idea is afforded by observations on the action of gum arabic solutions (in sea water) applied to eggs with exaggerated membrane elevation. In such cases, the membranes promptly collapsed back against the surfaces of the eggs.

Pasteels (1950) has also suggested that there may be a rhythmic and localized release of some sort of material from the cortex of the *Chaetopterus* egg, during the post-fertilization period. His experiments involving treatment of eggs of this form with KCl appear to support such an idea if one assumes that the KCl, like cold, blocks the process of release during the course of the treatment.

Pasteels was able to demonstrate a direct correlation in fixed KCl-treated eggs between areas of abnormal membrane wrinkling, and cortical and membrane alterations of structure and staining capacity. This correlation appears to be comparable to that observed in the present study of fixed and stained cold-treated eggs.

Exaggerated membrane elevation as a consequence of other experimental procedures

Lillie (1902) mentioned briefly the occurrence of exaggerated membrane elevation and dissolution in KCl- and $CaCl_2$-treated fertilized and unfertilized *Chaetopterus eggs*. A more thorough study of the same problem was undertaken by Pasteels (1950), who treated unfertilized *Chaetopterus* eggs with KCl by the method of Lillie. Pasteels illustrates a process of asymmetrical membrane elevation which, in some respects, is reminiscent of that reported in the present study. His Figure B shows that by 40 minutes after the end of KCl-treatment (95% sea water, 5% 2.5 *M* KCl, for one hour), there are the beginnings of an asymmetrical membrane elevation which, at this stage, is similar to that observed after cold-treatment. The subsequent course of events after KCl-treatment, however, is quite different; by 102 minutes after the end of this treatment, there is a very asymmetrical, deeply rugose exaggeration of membrane elevation. Apparently this stage is not succeeded by a smooth state of exaggerated membrane elevation.

Redfield and Bright (1921) reported exaggerated elevation of the *Nereis* egg membrane after various types of irradiation. Following beta or gamma radiation, the elevation was symmetrical; after alpha or ultraviolet treatment, it was asymmetrical. Redfield and Bright state that they did not obtain an increase in the volume of the perivitelline space after the irradiation of eggs such as *Cumingia*, *Asterias*, *Arbacia* and *Chaetopterus*, which do not normally produce jelly as a part of the fertilization reaction. However, Moser (1939), Spikes (1944) and Rustad (1959) reported the asymmetrical elevation of the sea urchin egg fertilization membrane after ultraviolet irradiation of one side of the eggs. It is of interest that x-irradiated unfertilized *Chaetopterus* eggs (treated with 20,000 and 40,000 r

and then inseminated) showed no exaggeration of membrane elevation (Henley, 1958). Furthermore, whole-mount preparations of such x-irradiated eggs, fixed at the metaphase of the first cleavage, do not reveal the asymmetry of cortical material reported above for cold-treated eggs. Although this observation is by no means conclusive evidence, it does suggest that the appearance of such cold-treated ova is associated with the postulated abnormal retention and delayed release of cortical material.

As noted in the introduction, alkaline sodium chloride treatment (pH 10.5) results in a drastic exaggeration of membrane elevation in the *Nereis* egg (Costello, 1945; Lovelace, 1949); this exaggeration, unlike that reported here, is symmetrical in nature, except for the circumscribed area of sperm entrance. Alkaline NaCl-treatment of *Chaetopterus* eggs is followed by an extremely rapid elevation of the membrane, which results in denuding of the eggs in less than five minutes. The exact process involved will be described in a later communication; suffice it to say here that the membrane elevation appears to be only superficially comparable to that produced as a result of cold-treatment.

Moser (1939) illustrates asymmetrical (although not especially exaggerated) membrane elevation in saponin-treated unfertilized *Arbacia* eggs.

The chronological relationships of events in cold-treated vs. control eggs

It is interesting to compare the chronological relationships of events in the cold-treated eggs and in the normal, untreated eggs. Pasteels (1950) has described the following series of membrane shape changes and wrinklings in fertilized *Chaetopterus* eggs; he did not specify the room temperature but, from the times noted for several "landmark" events, this may be assumed to have been about 17–20° C., somewhat lower than those prevailing during our experiments.

20 minutes after insemination: There is a vegetal-to-animal pole wave of shallow membrane wrinklings.
23 minutes after insemination: The first polar body is given off; from now until after the second polar body appears, the membrane remains smooth.
30 minutes after insemination: The second polar body is given off.
32 minutes after insemination: A second vegetal-to-animal pole wave of membrane wrinkling occurs.
38 minutes after insemination: "Pear-shaped" stage; there is now an animal-to-vegetal pole wave of wrinkling in the membrane, reversing the first two waves.
42 minutes after insemination: Polar lobe stage; wrinkling of the membrane now occurs in a wave from *each* pole, more or less simultaneously, leaving an equatorial band around the egg free of wrinkles.
66 minutes after insemination: First cleavage begins; the wrinklings of the membrane are accentuated.

Our observations showed that for all experiments, both with and without temperature shock, the greatest incidence of exaggerated membrane elevation occurred immediately before the first cleavage (at a stage corresponding to the polar lobe although, as noted above, the normal shape changes of the egg cytoplasm were often not recognizable in the treated eggs). This is a time, of course, when a number of

important events are occurring within the egg, in preparation for the first cleavage. It does not seem unreasonable to suppose that the double wrinkling (animal-to-vegetal and vegetal-to-animal) described by Pasteels as occurring shortly before the first cleavage, or some feature of this phenomenon, may be exaggerated to result in the reported events.

Summary

1. Fertilized eggs of the polychaete annelid, *Chaetopterus pergamentaceus*, were cold-treated for various periods of time, ranging from 150 to 720 minutes, beginning immediately after insemination. Two general methods were employed; in one, the eggs were plunged into pre-chilled, filtered aerated sea water (2–3° C.); these experiments are referred to as involving temperature shock. In the other type, the eggs were gradually chilled to approximately the above temperature. At the end of the treatment period all eggs were allowed to return gradually to room temperature.

2. When eggs were cold-treated, with or without temperature shock, there was a pronounced asymmetrical exaggerated elevation of the vitelline membrane, which reached its greatest incidence about 40 minutes after the end of treatment, or shortly before the first cleavage. This exaggerated elevation continued in some cases after prolonged cold-treatment, so that the eggs were eventually denuded.

3. Most of the cold-treatments used were followed by delays in the first cleavage time for 50% of the experimental eggs as compared with 50% of the control population.

4. In all cases where cold-treatment was initiated with temperature shock, the effects on membrane elevation and cleavage time were much more pronounced than when the eggs were chilled gradually.

5. A number of characteristic morphological and cytological abnormalities were noted in embryos developing from the treated eggs; these included severe ciliary defects, fragmentation of the embryos, lagging or lost chromosomes or chromosome fragments, duplication of chromosome sets and/or individual chromosomes, suppression of polar bodies, and multipolar spindles.

6. It is suggested that the findings reported here afford evidence supporting Costello's (1958a) hypothesis that the rhythmic wrinkling and shape changes reported for the normal *Chaetopterus* egg by Pasteels may be due to the gradual release of some colloidal material from the surface of the egg. This release is considered to be blocked in some manner by the low temperature, and when the treatment is terminated, the release proceeds in a drastic, non-rhythmic manner; it then appears to be coupled with a change in the permeability of the vitelline membrane, so that the colloidal material is retained between the egg surface and the membrane. This brings about the exaggerated membrane elevation observed.

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